EFFECTS OF STARVATION ON FREE AMINO ACIDS IN LARVAL BLOOD OF ORIENTAL BEETLE, ANOMALA ORIENTALIS WATERHOUSE[1]

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INTRODUCTION

Although it has been established that the nonprotein nitrogen concentration of insect haemolymph is high, the amino acids of this component have not been as thoroughly determined. With the development of the paper chromatographic method, data about the free amino acids of insect blood have increased (Auclair, 1953; Auclair and Maltais, 1954). It has been shown by Drilhon (1950), Auclair and Durbeuil (1953) and Micks (1956) that most of the naturally occurring amino acids are represented in the free state in insect blood.

While effects of starvation on body protein have been studied in certain insects (Slowtzoff, 1905; Heller, 1926; Lafon, 1941; Ludwig, 1950; Newton, 1954) there are few data pertaining to effects upon blood protein. Heller and Moklowska (1930) reported a 40 per cent decrease of blood protein in the moth, *Deilephila euphorbiae,* during starvation. Beadle and Shaw (1950) found that, while the plasma protein nitrogen of larvae of the neuropteron, *Sialis lutaria,* fell to 5 per cent of its original value, the nonprotein nitrogen (amino acids) remained constant during starvation. Analyses of the blood of starving Japanese beetle, *Popillia japonica,* larvae by Ludwig and Wugmeister (1953) showed that the blood protein nitrogen remained constant and the nonprotein and amino nitrogen components increased approximately two-fold.

The object of the present study was to note to what extent starvation affected the plasma amino acids of third instar Oriental beetle, *Anomala orientalis,* larvae.

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MATERIAL AND METHODS

Larvae of the Oriental beetle were collected in the field and brought into the laboratory. Each larva was placed, individually, in one-ounce ointment tins containing moist soil and a few grains of wheat. The larvae were allowed to feed for two weeks at 25°C., moisture and food being replenished, when needed. At the end of the feeding period the larvae were placed in individual vials in a desiccator, the base of which was filled with distilled water. Bellucci (1939) observed that under these conditions the water content of larvae remained constant. The larvae were starved for four weeks, analyses being made at the end of each week.

Blood was collected from normal and starved larvae, etherized to prevent blood coagulation, according to the procedure reported by Ludwig (1951). Pooled, whole blood collected from larvae was used for protein and nonprotein determinations by the micro-Kjeldahl technique of Koch and McMeekin (1924) and for amino nitrogen data by the photometric method of Russel (1944). The preparation of blood for chromatographic amino acid analysis was essentially that described by Pratt (1950). A detailed account pertaining to the separation, identification and concentration of the free and derived amino acids has been reported (Po-Chedley, 1956).

OBSERVATIONS

The protein, nonprotein and amino nitrogen values are shown in Table 1. The protein nitrogen values were relatively constant

TABLE 1

CHANGES IN THE COMPOSITION OF ORIENTAL BEETLE BLOOD DURING STARVATION (VALUES IN MILLIGRAMS PER CENT)

Degree of Starvation	Protein Nitrogen	Nonprotein Nitrogen	Amino Nitrogen
Normal	310.6	511.6	196.8
1 week	300.5	547.3	255.0
2 weeks	336.1	676.5	320.8
3 weeks	329.4	849.8	343.2
4 weeks	340.7	954.1	350.7

during the four weeks of starvation, the weekly reports approximating the normal average of 310.6 mg. per cent. The nonprotein nitrogen rose steadily from a normal average of 511.6 to a final average of 954.1 mg. per cent. There was a similar increase of amino nitrogen from a normal average of 196.8 to a terminal average of 348.7 mg. per cent.

TABLE 2

CHANGES IN THE AMINO ACIDS OF THE HAEMOLYMPH OF ORIENTAL BEETLE LARVAE DURING 4 WEEKS OF STARVATION (VALUES IN MG. PER CENT AMINO NITROGEN)

Compound	Normal	1 week	2 weeks	3 weeks	4 weeks
alpha-alanine	10.8	12.7	18.8	21.6	22.6
alpha-n-amino butyric acid	5.4	5.7	9.7	9.7	10.6
arginine	4.6	5.6	8.2	10.4	11.0
asparagine	3.1	3.4	5.0	1.8	1.5
aspartic acid	4.5	5.0	10.1	13.6	15.3
beta-alanine	3.7	4.4	7.1	7.5	7.7
cystine	1.8	2.1	2.8	2.9	3.0
glutamic acid	6.7	7.3	12.8	14.5	19.5
glutamine	12.4	14.3	18.9	7.4	4.4
glycine	33.1	51.1	68.8	73.4	76.6
histidine	4.8	4.9	7.6	8.6	8.8
isoleucine	10.6	12.2	16.2	17.4	17.4
leucine	7.5	7.7	10.3	11.7	11.5
lysine	7.9	9.3	12.3	13.7	15.5
methionine	5.4	5.8	8.7	9.3	10.0
ornithine	2.5	2.9	5.6	5.8	6.1
phenylalanine	10.4	10.9	11.4	12.3	12.0
proline	2.7	3.8	6.8	7.7	8.2
serine	2.4	3.8	6.0	7.9	8.1
taurine	4.8	5.1	5.4	5.7	6.6
threonine	3.6	4.0	5.4	6.5	8.8
tryptophane	14.6	14.7	18.7	20.2	22.3
tyrosine	12.3	13.4	12.1	6.6	2.2
valine	4.2	4.8	7.8	9.2	10.6
Total	179.8	214.9	296.5	305.4	320.8

The 21 free amino acids and 3 derivatives determined in the blood of this insect and total amino nitrogen values are listed in Table 2. The compounds which occurred in highest concentration in normal larvae are glycine, alpha-alanine, glutamine, isoleucine, phenlyalanine, tryptophane and tyrosine. All of the amino compounds, with the exceptions of the amides and tyrosine, increased during the period of inanition. The tyrosine concentration of 12.3 mg. per cent for normal larvae increased to 13.4 by the end of the first week and subsequently decreased to 2.2 mg. per cent at the end of the fourth week. The amides, asparagine and glutamine, increased during the first two weeks before decreasing to their final concentrations of 1.5 and 4.4 mg. per cent, respectively. The amino acids, isoleucine, leucine, arginine and phenylalanine, were relatively constant between the third and fourth weeks of starvation. Glycine existed in highest concentration during all analyses, whereas, cystine was consistently low.

DISCUSSION

The results of this study, which indicate that the blood protein is relatively constant in this insect during starvation, agree with the findings of Ludwig and Wugmeister (1953) for Japanese beetle larvae. The stability of blood protein in Oriental beetle larvae suggests its replacement by extravascular protein at a rate equal to its utilization. The increased amino nitrogen concentration in haemolymph of Oriental beetle larvae during starvation augments Newton's (1954) study. In that investigation of total nitrogen in starving Japanese beetle larvae the shift in nitrogen was represented by the increase of amino acids and nitrogen end-products in the blood.

It is apparent from Table 2 that the amino nitrogen of this insect is represented by a wide variety of amino acids and their derivatives, which, with three exceptions, increased in concentration during inanition. Certain phenomena, which occurred during starvation, may be interpreted on the basis of amino acid changes. The decrease in tyrosine concentration was anticipated because of observations on the blackening of blood during the bleeding process. The blood of normal and partially starved larvae usually darkens rapidly, when exposed to air, indicating the catalytic action of tyrosinase upon tyrosine to produce a melanin. This color was not as intense during the late weeks of starvation at which time the tyrosine concentration had decreased to 2.2 mg. per cent. In addition the larvae were also paler than normal at this period. This is in accord with the findings of Golberg and De Meillon (1948) who found that both tyrosine and phenylalanine influence the pigmentation of the mosquito, *Aedes aegypti.* The phenylalanine concentration of the Oriental beetle larvae, which was relatively constant during this interval, could not, apparently, compensate for the tyrosine depletion.

The increased concentration of the amino acids, aspartic and glutamic, may be due to the conversion and decrease of asparagine and glutamine as suggested by Ussing (1946). The high concentration reported for glycine as well as the general increase for the amino acids would appear to result from the general diminished metabolic rate existing during starvation (Bellucci, 1939). In this respect Kutscher and Ackermann (1933), referring specifically to glycine, contended that the rapidity of insect metabolism prevented this acid from accumulating in high concentration in the blood. The increase of arginine during inanition suggests muscle proteolysis with the release and subsequent decomposition of phosphoarginine. Cystine values which were relatively constant indicate a retardation of its function as an important agent for moulting (Golberg and De Meillon, 1948). Similar unpublished data for the mealworm, *Tenebrio molitor,* show that both cystine and tyrosine increase in concentration prior to ecdysis and are present at a reduced level following the moult.

The two-fold increase of the free amino acids, which was observed, does not completely explain the elevated nonprotein nitrogen concentration. It may also depend on the discharge of other nitrogenous compounds, such as glucosamine, urea and various purine derivatives into the haemolymph.

SUMMARY

1. Oriental beetle, *Anomala orientalis,* larvae were starved 4 weeks. Determinations were made on the blood each week to establish changes occurring in the protein, nonprotein, amino nitrogen and free amino acid concentrations.

2. Protein nitrogen values were relatively constant during the entire period of study. Nonprotein and amino nitrogen concentrations increased approximately two-fold during the same period.

3. Twenty-one free amino acids and three derivatives were

identified by the paper chromatographic method in the blood of this insect.

4. All of the identified compounds, except tyrosine, asparagine and glutamine, increased in concentration throughout the four weeks of starvation.

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closed and the Secretary empowered to cast one ballot for the election of these officers.

Upon completion of the above business, President Dr. Asher Treat took the Chair and introduced the speaker of the evening, Dr. Roman Vishinac, who addressed the gathering on the topic: "Man in the world of Nature."

Dr. Vishniac presented his talk in essentially two parts, the first being an introductory discourse on the complexity of living things and the second being a showing of his remarkable Kodachrome slides of organisms illustrating this complexity.

The study of Biology as a science is always in a state of flux and our ideas of the nature and relationship of living things abruptly change from generation to generation. The earlier concept of classification was quite different from that of today. Living things and natural phenomena are now considered much more complicated than heretofore. The basic views are now being subjected to clarification and we have moved forward another step—that to question the origin of life itself.

The simplest living matter known today—protoplasm—is extremely complex, being made up of a systematic aggregation of numerous organic substances. How these more basic substances came into being is now under consideration. At this point Dr. Vishniac gave a brief resume of the various theses purporting to explain the origin of living matter in nature. Through experimental means some light has been thrown on the subject. Nitrites may be synthesized into amino acids by the use of ultra-violet light; in the process of cooling and condensation the miasma, during the period of the formation of the earth, carbon in the form of carbides or acetylene could possibly form nucleic acids, the building blocks of living matter; by the use of the cyclotron several isotopes of carbon may be formed from simple carbon; electrical discharges might bring about a polymerization of carbon and thus produce complex molecules; by subjecting simple basic substances to very high temperatures and pressure, complex end-products may be produced. All these are possibilities in explaining the

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